

Open Literature Review Summary

Chemical Name: Imidacloprid

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Kirchner, W.H. 2000. The effects of sublethal doses of imidacloprid, hydroxy-imidacloprid and olefin-imidacloprid on the behavior of honeybees. Unpublished Study Report.

Purpose of Review (DP Barcode or Litigation): N/A

Date of Review: 04/02/12

Summary of Study Findings:

Summary: The effects of imidacloprid and two of its metabolites (olefin and dihydroxy-imidacloprid) were studied in laboratory and field settings on honeybees. In the field, sucrose solutions containing the olefin metabolite were fed to foragers and foraging and communication behavior were analyzed. The behavioral effect of the olefin is similar to imidacloprid though they occur at relatively higher concentrations. Observed effects from the olefin were an increase in the frequency of tremble dances, but no disorientation could be found and no significant effect on foraging activity up to 100 ppb. Imidacloprid and its two metabolites were assessed using the proboscis extension reflex. Imidacloprid reduced learning performance at 100 ppb, but not at 50, 20, or 10 ppb. Both of the metabolites did not affect the learning performance at 100 ppb. However, effects at 500 ppb were found with olefin and at 2 ppm with the dihydroxy imidacloprid. In addition, long-term effects of feeding sucrose solutions containing 10ppb imidacloprid to young bees kept for 10-12 days showed no effect on learning performance in the proboscis extension reflex.

Methods: The experiments used two honeybee colonies of the strain, *Apis mellifera carnica*, in Konstanz, Germany, during the summer of 1999. One colony of about 5000 bees was set up in a two frame observation hive for the communication experiments in the field. The second colony of about 20 – 30 K bees provided bees for the laboratory experiments on olfactory learning performance. Imidacloprid was obtained (M00680, 99.4%) and 100.6 mg were dissolved in solution to produce a solution of 100 ppm. After dilution, a stock solution of 2 ppm was made. The feeding solutions were made by dissolving either 1 mol or 2 mol sucrose in water, adding the stock solution, and then filling up to 1L, resulting in concentrations of 1M or 2M and a range of imidacloprid concentrations. In a similar fashion, 105.2 mg of dihydroxy-imidacloprid (95%) was diluted to obtain the same feeding solutions. 10.2 mg of olefin-imidacloprid was dissolved in ethanol and diluted to make the feeding solutions in a similar manner as the parent compound.

For the field experiment, groups of individually marked bees were trained to visit an artificial food source located 500m from the observation hive. At the feeder, the 2M sucrose solution was provided. Tests were performed on olefin-imidacloprid at

concentrations of 10 ppb, 20 ppb, 50 ppb, and 100 ppb. Foraging activity was recorded for marked individuals at the feeding site. At the hive, the behavior of returning foragers was captured on video using an infrared video camera under dim red light that is invisible to bees. The directions indicated by the waggle dances in the hive were analyzed from the infrared video tapes to the nearest 1° and the distances were identifiable by the duration of the tail-wagging movements to the nearest 20m.

For the laboratory experiment, the proboscis extension reflex (PER) was used to evaluate learning performance. Bees were caught on approach to an artificial feeder in the field, cooled, and harnessed in plastic tubes. In this manner, only the mouth parts were free to move, including the proboscis and the antennae. The animals were initially tested for the unconditioned PER by touching the antenna with sucrose solution. Only animals that showed a response were used for the test. Groups of 20 bees were set-up in a carousel with 20cm distance between individuals. This carousel was turned around to place one bee after the other in front of an olfactometer 2cm away from the bees antennae and an exhaust above the test animal. Peppermint oil was used as the test odor and was provided by loading 5 µL of odorant on a strip of filter paper placed into a 1 mL plastic syringe which was loaded into the olfactometer. The flow of air containing the odor was directed against the antennae of the bee for 6 seconds. To condition the bees, the antennae were briefly touched with 1M sucrose solution on a glass rod three seconds after the onset of the odor pulse and the bee was then rewarded for 3 seconds. PER observed within the first 3 seconds of odor delivery were considered conditioned responses. Odor pulses during the test trials of 6 second duration were supplied and proboscis extension within 10 seconds after the onset of the odor pulses were scored as a conditioned responses.

Short term effects were evaluated using PER for imidacloprid, dihydroxy-imidacloprid, and olefin-imidacloprid. The compounds were fed to the bees only during the training. Imidacloprid was tested at concentrations of 10, 20, 50, and 100 ppb in 1 M sucrose solution. Olefin-imidacloprid was tested at concentrations of 100 ppb and 500 ppb. Dihydroxy-imidacloprid was tested at 100 ppb and 2 ppm in 1 M sucrose solution. A solution containing 0.5% ethanol and 1 M sucrose was used for the control group and in experiments with the olefin.

Long-term effects were only tested with parent imidacloprid. For the long-term effects, combs containing capped brood were kept in an incubator at 34°C. Groups of 50 hatched bees each were placed in plastic containers in an incubator at 30°C and allowed to feed ad libitum on 1 M sucrose solution containing 10 ppb imidacloprid for 10-12 days. In addition, water and pollen were supplied ad libitum. The bees were then tested for unconditioned and conditioned responses as described previously.

Results: Within the range of tested concentrations of the olefin metabolite, the study did not reveal any effect of the olefin metabolite on the probability of waggle dances (dances that recruit foragers to a food source) up to 100 ppb (Figure 1). However, the study author reported that the olefin did significantly increase the number of foragers engaging in tremble dances (dances that inhibit forager bees to a food source) (Figure 2). However, the study author did not state at which concentration the increase was statistically significant, but from the figure the 100 ppb elicits the greatest magnitude of effect. The frequency of visits of individually marked foragers at the feeding site at 500 m from the hive was weakly but not significantly affected by the olefin metabolite but

only at 100 ppb. All data in Figures 1, 2, and 3 related to imidacloprid are from a previous study.

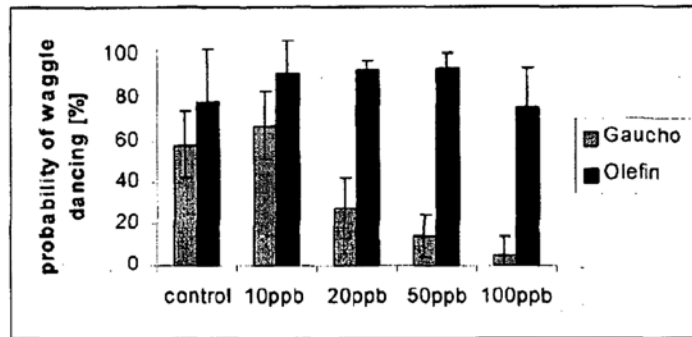


Fig. 1: Probability of waggle dances of individually marked forager bees returning from artificial feeding sites at a distance of 500m from the hive providing 2M sucrose solution containing imidacloprid or olefine-imidacloprid at the concentrations indicated. Means and standard deviations of 4-6 tests each on a total of about 800 bees. Whereas the frequency of waggle dancing decreases significantly at imidacloprid (gaucho) concentrations of 20 ppb and higher (data from the 1998 series of experiments) no effect could be found with the olefine-imidacloprid up to 100 ppb.

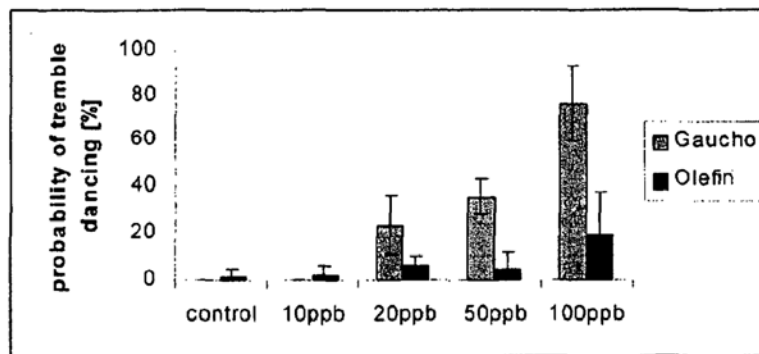


Fig. 2: Probability of tremble dances of individually marked forager bees returning from artificial feeding sites at a distance of 500m from the hive providing 2M sucrose solution containing imidacloprid or olefine-imidacloprid at the concentrations indicated. Means and standard deviations of 4-6 tests each on a total of about 800 bees. The frequency of tremble dancing increases significantly when bees had fed on sucrose solutions containing imidacloprid (gaucho) concentrations of 20 ppb and higher (data from the 1998 series of experiments). The effect was found to be significantly weaker with the olefine-imidacloprid in the same range of concentrations.

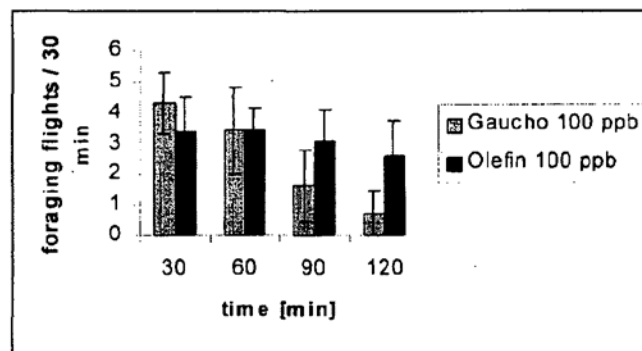


Fig. 3: The number of visits to a food source located 500m away from the hive decreased significantly during a two-hour observation interval when 2M sucrose containing imidacloprid at 100 ppb was provided, but decreased only insignificantly when olefine-imidacloprid was provided at the same concentration. The graph shows the number of foraging flights per forager per 30 min. Mean values and standard deviations of 40-100 bees.

There did not appear to be any effect of the olefin metabolite at the concentrations tested on bee communication of distance and directional information.

In short term tests, imidacloprid fed to bees affected the conditioning rate only at 100ppb. With the olefin metabolite, there was a reduced learning performance at 500 ppb or 2 ppm dihydroxy-imidacloprid. For the study on long term effects, no differences were detected between imidacloprid and the control groups. However, the learning performance was generally highly variable between batches of bees (controls as well as test bees) and the overall performance was relatively low suggesting that the long term part of the study needs refinement in the methodology prior to use of the results.

Description of Use in Document (QUAL, QUAN, INV):

Qualitative

Rationale for Use: This study presents useful information on the relative sublethal toxicity of the olefin, dihydroxy-imidacloprid metabolites, and parent imidacloprid related to learning ability. The study also presents useful information on the range of concentrations up to 100 ppb for the olefin metabolite to affect the type and accuracy of communication of bees in the hive. The results show that the olefin and dihydroxy-metabolites can also affect learning performance of bees but at much higher concentrations than the parent compound, over an order of magnitude difference.

Limitations of Study: Forager bees were used for the experiment related to the short term learning ability and the field portion of the study. There is uncertainty as to what the effect may be of imidacloprid and metabolites on learning ability for younger bees. The study tried to evaluate the effect on younger bees, but the study author states that the overall performance was low. The study author stated that all of the test solutions were kept in the dark at 4°C. When bees were trained to visit feeders providing solutions containing imidacloprid, these feeders were never exposed to direct sunlight, and the solutions were exposed to ambient temperature for a maximum of two hours. The study

report does not evaluate changes in the concentration over time during the field portion. The stability of the material could not be evaluated during the field portion of the experiment, nor in laboratory experiment. Statistical significance was not reported for some of the endpoints.

Other details are missing from the study report. It does not state if the field study portion was conducted all on the same day, or on different days for the treatment groups. In addition, for the field study portion of the study, the highest concentration tested was 100 ppb for the olefin-metabolite. Consequently, the study author could not establish an effect threshold on the communication of bees to the food source, only the number of tremble dances, which represents an inhibition of recruitment to a food source. But the study author does not state at which concentration there is a significant effect. Also, for the laboratory part of the study, the author does not state that the prothoracic legs were free and allowed to move so that the honeybee could clean its antennae from the repeated sucrose stimulations. This may introduce uncertainty into the results due to repeated stimulations and the inability of the bees to clean their antennae.

Primary Reviewer:

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